"A STUDY ON POTENTIAL OF *Lasiurus scindicus* IN BIOTREATMENT OF INDUSTRIAL EFFLUENTS IN JAIPUR"

Research Protocol

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INTRODUCTION

Environmental contamination with heavy metals is a global disaster that is related to human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agricultural, sludge dumping and melting operations. All the heavy metals with high concentration have strong toxic effects and are regarded as environmental pollutants [1]. Metal pollution has become one of the most severe environmental problems today. Excessive accumulation of heavy metals is toxic to most plants. The most common heavy metals contaminants are Cd, Cr, Hg, Pb, Cu and Ni. Heavy metal ion when present at an elevated level in the environment, are excessively absorbed by roots and translocated to shoot, leading to impaired metabolism and reduced growth [2-3]. Excessive metal concentration in contaminated soil results in decreased soil microbial activity and soil fertility and yield loses [4]. Metals deposited to soil may transform (solubilize) into more mobile forms capable of migrating into the soil water where they may pose an elevated risk to plants, other soil biota and to ground water [5]. The problem of environment either directly or indirectly: waste generation (sewage, waste water, kitchen waste, industrial waste, effluents, agricultural waste, food waste) and use of chemicals for various purpose in the form of insecticides, pesticides, chemical fertilizers, toxic products and by- products from chemical industries. The more serious concern is the increased presence of toxic chemicals such as halogen aliphatics, aromatics, polychlorinated biphenyls and other organic pollutant which may reach air, water or soil and affect the environment in several ways, ultimately threatening the self-regulating capacity of the biosphere [6]. The research aim in recent years obtain methods to be appropriate environmental and cost effective for the clean-up of contaminated soil and water. Bioremediation is becoming an increasingly popular and sustainable alternative over conventional methods for treating wastes and contaminated media in view to degrade and ultimately stabilize the contaminants using microbial activity. Society is looking for ways to minimize these impacts or avoid them altogether, so that human activity can become more sustainable. The technologies for remediation of contaminated land and waters are beneficial as they reduce the risk from contamination and are unlikely to do much other harm. Bioremediation methods are

generally categorized into ex-situ and in-situ bioremediation. In the *in-situ* and *ex-situ* bioremediation techniques namely anaerobic digestion technology, phytoremediation, composting, bioaugmentation, biostimulation and biosorption for their effectiveness in the biotreatment, stabilization and eventually overall remediation of contaminated strata and environment.

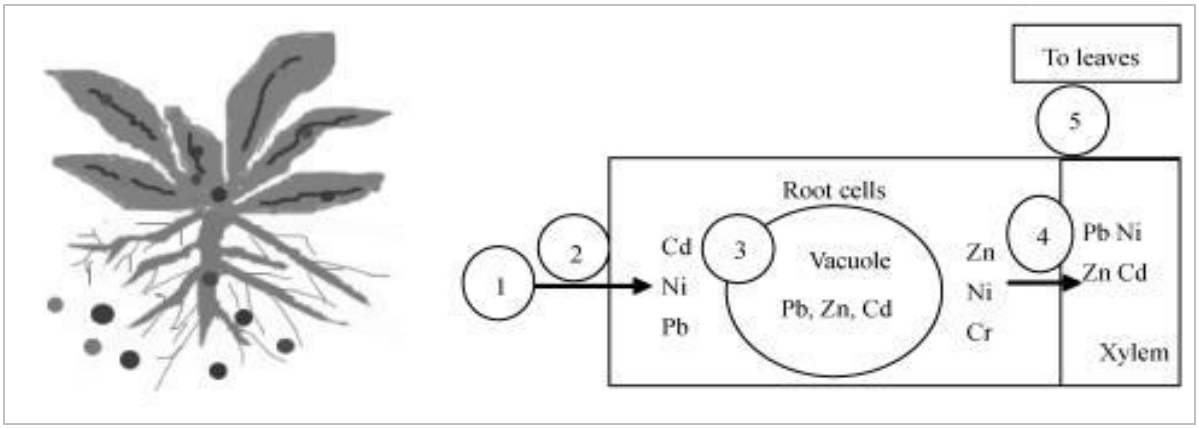
Ex-situ bioremediation technologies are those which involves the physical removal of the contaminant to another area (possibly with in the site) for treatment. Bioreactors, land farming, anaerobic digestion, composting, biosorption and some other forms of solid phase treatment are all examples of ex-situ treatment techniques.

In-situ bioremediation involves treatment of the contaminated material in place. Bioventing and biostimulation treatment of contaminated soil by indigenous aquifer microorganism are example of these treatment technology [7].

Untill now, methods used for heavy metal contaminated soil remediation such as excavation and land fill, thermal treatment, acid leaching and electroreclamation are not suitable for practical application, because of their high cost, low efficiency, large destruction of soil structure, soil properties and site condition. Thus the development of Phytoremediation strategies for heavy metals contaminated soil is necessary [8-10] . In present study we are focusing on one of the ex-situ technique of bioremediation by using *Lasiurus scindicus* on waste water effluent coming in Sitapura industrial area Jaipur. Jaipur (Longitude: 95^0 24' E ;latitude:$27^0$18' N) is a city located in the central part of the rajasthan is undergoing rapid urbanization and industrialization. Almost in all parts of Jaipur city and adjacent areas, a large number of industries have come up during last two decades. Such area includes Vishwakarma, Sudershanpura, Bais Godown, Jhotwara, Malviya, Sanganer, Sitapura industrial areas, etc., which play a major role in polluting different water resources. All the wastes from domestic, municipal and industries are discharged in Amanishah Nala., which is the largest element of drainage system of this city and covers the whole city. The farmers use this water for growing vegetables and other crops. This direct use of the wastewater by the farmers necessitates evaluation of the quality of the water to determine the effects on the fertility

of the soil and quality of the vegetables grown on it. Amanishah Nala receives industrial and domestic wastes from various sites and seepage of this water affects groundwater. Although, application of sewage effluents was reported to be beneficial in increasing crop yield and reducing fertilizer requirement [11], but some other studies showed that metals like Cu(copper), Cd(cadmium), Cr(chromium) enter the food chain through their application in soil and this ultimately causes health problems significantly [12]. Therefore, the present study is intended to characterize the wastewater of sitapura industrial area. In present study we are focusing on industrial effluent near sitapura industrial area. Phyoremediation is the process to clean up the environment. The word Phytoremediation comes from the greek word *phyto,* meaning “plant” and the latin word *remediare* meaning “to remedy”. This word is generally used to describe any system where plants are introduced into an environment to remove the contaminant from it. Phytoremediation is done in varieties of ways. The plants can be introduced into an environment and allowed to absorb contaminant into its leaves and roots. These plants can then be harvested and treated as hazardous waste. Phytoremediation can and has been used to clean up metals, pesticides, solvents, explosives, crude oil, polyaromatic hydrocarbons, land fill leachates, agricultural runoff, acid mine drainage, and radioactive contamination. Phytoremediation is an environmental friendly, safe, cheap way to clean up contaminants.

Phytoremediation of elemental pollutant is differing from the phytoremediation of the organic pollutant in the strategies used to remediate them. Phytoremediation of elemental pollutant involves extraction and translocation of metals to above ground tissues, to concentrate them in harvestable land parts, transformation of the element to less toxic form, stabilization or to sequester element in roots to reduce the mobility and bioavailability of pollutant in the environment [13]. Phytoremediation of organic pollutant involves transformation of organic pollutant into relatively non- toxic from such as carbon dioxide, nitrate, chlorine and ammonia. Two different strategies are used for the phytoremediation of organics- 1. Direct Phytoremediation by Plants (plants release some enzyme into soil and water that can degrade organic contaminants) and 2.Rhizosphere biodegradation by fungi and bacteria.



Metal transfer in plants:- 1: A metal ion is sorbed at root surface; 2: Bioavailable metal moves across cellular membrane into root cells; 3: A fraction of the metal absorbed into roots is immobilized in the vacuole; 4: Intracellular mobile metal crosses cellular membranes into root vascular tissue (xylem); 5: Metal is translocated from the root to aerial tissues (stems and leaves)

There are five different types of phytoremediation mechanism for contaminant removal (organic/ inorganic).

Phytotechnology	Mechanism	Pollutants
1. Phytoextraction	Hyperaccmulation in harvestable parts of plants	Inorganic: Co, Cr, Ni, Pb, Zn, Au, Hg, Ag, Mo, Cd Radionuclides: Sr, Cs, Pb, U
2. Rhizofilteration	Rhizosphere accumulation though sorption, concentration and precipitation	Organic/ inorganic: metals like Cd, Cu, Ni, Zn, Cr Radionuclides
3. Phytovolatilization	Volatilization by leaves through transpiration	Organic/ inorganic: Chlorinated solvents, inorganic (Se, Hg, As)
4. Phytodegradation	Pollutant eradication	Organic compounds, chlorinated solvents,

		phenols, herbicides, munitions
5. Phytostabilization	Complexation, sorption and precipitation	Inorganics: As, Cd, Cu, Cr, Pb, Zn, Hs

Part Literature Review :-

Phytoremediation, the use of plant to extract, sequester and/or detoxify pollutants through physical, chemical and biological processes.[14-16]. It has been reported to be effective, in-situ, non-instrusive, low cost, aesthetically pleasing, ecologically benign, socially accepted technology to remediate polluted soils [17-19]. The ideal plant for phytoremediation should grow rapidly, produce a high amount of biomass and be able to tolerate and accumulate high concentration of metals in shoots. Most of the commonly known heavy metals (Cd, Cr, Cu, Hg, Pb and Ni) accumulators belong to the Brassicaceae family [20]. The goal of soil focused phytoremediation often has to do with the presence of heavy metals such as nickel (Ni) lead (Pb), mercury (Hg), copper (Cu), zinc (Zn), chromium (Cr), aluminum (Al) cadmium (Cd) and the metalloid arsenic(As) [21].The sources of these toxins include metal working, coal combustion, sewage sludge, pesticides, and fertilizers. Other methods for remediation include excavation, soil washing, thermal treatment, electro-reclamation, chemical, and other biological techniques. However, all of these tend to be more expensive than phytoremediation. Heavy metals may take several forms. Methyl mercury is the most dangerous form of Hg and is typically created in an anaerobic environment such as the bottom of water bodies. It may then enter the food chain and bioaccumulate through fish. Lead is one of the most worrisome metals and it can be ingested through inhalation or ingestion. Lead can cause a vast array of neurological disorders especially in children. However, Zinc and Cadmium tend to be more bioavailable in soil than lead [22]. Although hyperaccumulators plants have exceptionally high metal accumulating capacity. Most of these have a slow growth rate and often produce limited amount of biomass when the concentration of available metal in the contaminated soil is very high. An alternative is to use species with a lower metal accumulating capacity but higher growth rate, such as

Indian mustard (*Brassica juncea*), another alternative is to provide them with an associated plant growth promoting rhizobacteria, which also is considered to be an important component of phytoremediation technology [23]. A hyperacculmulator is a plant species capable of accumulating 100 times more metal than a typical plant [24]. Typically hyperaccumulators will take up greater than 1% of their mass in metals [25]. Hyperaccumulator plants show a resource for remediation of metal contaminated sites, as they are able to extract wide range of metals and to concentrate them in their upper parts with the character of metal tolerance [26] . Most hyperaccumulators that have been identified take up Ni (nickel) while some have been shown to accumulate Pb(lead), Cd(cadmium), Co(cobalt), Cu(copper), Se(selenium), and Zn(zinc). Hyperaccumulators for Hg have not yet been found. Plant family trends may elucidate patterns in hyperaccumulation. Research shows that the mustard (Brassicaceae) and carnation (Caryophyllaceae) families contain many obligate metallophytes but are usually nonmycorrhizal. *Poinsettia* (Euphorbiaceae) and *Snapdragon* (Scrophulariaceae*)* families have also been shown to accumulate metals. Metallophytes in the subtropical and tropical parts of the world often belong to Bean (Fabaceae), Mint (Lamiaceae), Sunflower (Asteraceae) and Grass (Poaceae) families [27]. *Lepidium sativum* commonly called ‘‘Garden Cress’’ is a polymorphic species [28] . It is a native plant of South West Asia, which spread many centuries ago to western European. It was used by ancient Egyptians as a food source and became well known in various parts of Europe, including Britain, France, Italy and Germany in due course, where it is still used as a minor crop. Persian used to eat this plant even before bread was known [29]. *Lepidium sativum* is a plant that is used for phytoremediation of soil heavy metals contaminated [30-32]. Most plants show accumulation, tolerance or hyperaccumulation of only one metal. Water plants show the largest ability to accumulate multiple metals. These include Hydrilla (*Hydrilla verticillata*), Duckweed (*Lemna minor*), Water Lettuce (*Pistia stratiotes*), Water Fern (*Salvinia molesta*), Giant Duckweed (*Spirodela polyrhiza*), Water Hyacinth (*Eichhornia crassipes*), Water Hyssop (*Bacopa monnieri*), Water Fern (*Azolla filiculoides*) and Tape Grass (*Vallisneria americana*). Most of these are exotic to North America with the exception of Duckweed and Tape Grass. Many have escaped

cultivation and are now terrible problems all over the world. Several edible plants have been shown to accumulate metals. Water Hyacinth is edible however care should be taken not to consume it when metal contamination is a concern [33]. Some common edible terrestrial plants also have been shown to accumulate multiple metals including Indian mustard (*Brassica juncea*), Sunflower (*Helianthus annuus*) Bent Grass (*Agrostis castellana*) and Alpine Penny Cress (*Thlaspi caerulescens*). In our research we are focusing on waste water effluent of the sitapura industrial area in jaipur, where a large number of industries are situated and play a major role in polluting different resources. All the waste from domestic, municipal and industries are discharged in "Amanishah nala" ,which is the largest element of drainage system of this city. But we are focusing only on the effluent of sitapura industrial area where various industries (like textiles industries, marble industries, pharmaceuticals, soap industries, automobiles, chemicals, plastic industries, edible oils, LPG bottling plant,food products and other miscellaneous categories) effluent discharge into the "Amanishah Nala". The aim of my study is to study the potential of *Lasiurus scindicus* for biotreatment of waste water effluents from jaipur. *Lasiurus scindicus* is commonly known as sewan grass, karera, gorkha. Sewan grass *(Lasiurus scindicus* Henrard *(Poaceae)* is a perennial grass that can live up to twenty years. It is a bushy multi-branched desert grass with ascending to erect wiry stems, up to 1-1.6 m tall and a stout woody rhizome. Leaves are alternate with a thin leaf blade. The fruit is caryopsis. Sewan grass forms bushy thickets in sandy deserts where it is used for pasture, hay and fodder for livestock. The grazing pasture is of outmost importance in areas where annual rainfall is below 250mm. Sewan grass is one of the most important grazing grasses in north-west India (Rajasthan) and northern Pakistan in area with less then 250 mm of rainfall [34]. Sewan grass is very important in arid environment because they provide forage which maintains both wild mammals and livestock and soil cover. Sewan grass may be used to stabilize desert sandy dunes.

Statement of the Research Problem :-

"A Study on potential of *Lasiurus scindicus* in biotreatment of industrial effluents in Jaipur".

Objectives of the study :-

1. To collect water and soil samples from the waste water effluents.
2. To analyse collected water and soil samples for different parameters and heavy metals presence.
3. To study morphological and biochemical parameters and analyzing the role of *Lasiurus scindicus* in biotreatment of waste water.
4. To recommend the best technology for biotreatment.

Scope of the study :-

Due to industrial revolution, pollution by toxic metals has accelerated dramatically. So, the potentiality of such metals tolerant plant is aimed for biotreatment of polluted water and soil.

Researcher will analyse water and soil samples from Sitapura industrial area and study the effect of water and soil on morphology and biochemistry of *Lasiurus scindicus* plant to recommend the best technology for biotreatment.

Significance of the study :-

Contamination of industrial effluents in water and causing pollution is a very big problem now a day. Their removal is a big challenge as well as the need of the day. Biotreatment of industrial effluents contaminants by *Lasiurus scindicus* will be an initiative, which will provide us a new cost effective and eco-friendly method.

Lasiurus scindicus is one of the most important grazing grass in Rajasthan. If this study will be successful then it can provide multiple environment benefits by improving waste water quality and additionally providing valuable feed stock resources. And in future *Lasiurus scindicus* can be used as filter strips adjacent to waste water streams.

Limitations :-

- The study will be restricted only to waste water effluents of sitapura industrial area, Jaipur.
- The experiment will be confined only to the laboratory level.

Methodology:-

Following procedure will be followed for the experimentation:-

Collection of soil and water samples: soil samples will be collected in sterile plastic bags while water sample will be collected in plastic bottles from the industrial effluents of SIA jaipur from equal distances.

Analysis of water and soil samples: Soil and water samples will be subjected to the physiochemical analysis like pH, dissolved oxygen, biological oxygen demand, chemical oxygen demand, chloride, hardness, calcium, phosphate, magnesium, sodium, potassium, organic matter [35-36]. The detection of heavy metals presence will be done in two steps:-

1. Presumptive test 2. Confirmative test

Presumptive test will be done by Osumex kits for the heavy metals detection. After the presumptive test samples will be subjected to atomic absorption spectrophotometer (AAS) for confirmative presence of heavy metals [37-38].

Then we will do Phytoremediation of water and soil samples by using two techniques:

1. Thin film technique 2. Mechanistic method

1.Thin film technique: in this technique biotreatment of contaminants by *Lasiurus scindicus* seeds will be put in hydroponically developed system containing waste water. Approximately 10 liter of effluent will be brought to the laboratory and experiment will be set up in plastic pots. one of the pot will be filled with concentrated effluent (100%) and then its dilution will be done accordingly (80%, 60%, 40%, 20%) and then transferred to

the pots. control will be maintained. Seeds of *Lasiurus scindicus* will be sown in a pot and after germination thining of plant will be done. Approximately five plants in each pot will be transferred in the hydroponic system developed with above mentioned dilutions. The experiment will be set up in replica of three. For the morphological and biochemical studies, the *Lasiurus scindicus* plant will be periodically uprooted at a time interval of 7, 14,21,28, 35 days. The quantification of heavy metals ions in industrial effluent will be performed on Atomic Absorption Spectrophometer (AAS).

2.Mechanistic method : In this method approximately 1 kg of sterile soil sample will be collected at a depth of 0-15 cm after scrapping the top 1cm layer of soil. The collected soil will be stored in sealed plastic bags and transported to the laboratory. For the experimentation soil samples will be put in plastic pots. Approximately 5 seeds of *Lasiurus scindicus* in each pot will be grown for study and irrigated by the waste water effluents. As mentioned above method dilution of waste water effluents will be done (100%, 80%, 60%, 40%, 20%). Control will be maintained. Periodically morphological and biochemical parameters will be studied by harvesting the plant at a time interval of 7, 14, 21, 28, 35 days respectively. The quantification of heavy metals ions by Atomic Absorption Spectrophotometer (AAS).

Month wise plan of work

1-24 months	Review of literature throughout the study
1-3 months	Samples collection
3-8 months	Analysis of water and soil sample
8-14 months	Morphological and biochemical characterization of *Lasiurus scindicus*
14-20 months	Analysis and interpretation of results
20-24 months	Thesis preparation

Total Time (Expected): 24 Months

Work schedule (Months wise)

Activity / Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Review of literature throughout the study																								
Samples collection																								
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